

PATENT COOPERATION TREATY

REC'D 28 OCT 2005

WIPO

PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/EP2005/051464

International filing date (day/month/year)
31.03.2005

Priority date (day/month/year)
02.04.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/90, C12N5/00

Applicant
DSM IP ASSETS B.V.

1. This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the international application
- Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for International preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Guarinos Viñals, E

Telephone No. +49 89 2399-7228



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/051464

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - a sequence listing
 - table(s) related to the sequence listing
 - b. format of material:
 - in written format
 - in computer readable form
 - c. time of filing/furnishing:
 - contained in the international application as filed.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority for the purposes of search.
3. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. II Priority

1. The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/051464

Box No. III Non-establishment of opinion with regard to novelty, inventive step and Industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

the entire international application,
 claims Nos. 1-3, 10, 11, 16

because:

the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 the claims, or said claims Nos. 1-3, 10, 11, 16 are so inadequately supported by the description that no meaningful opinion could be formed.
 no international search report has been established for the whole application or for said claims Nos.
 the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form

has not been furnished
 does not comply with the standard

the computer readable form

has not been furnished
 does not comply with the standard

the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/051464

**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or
industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-17, 24, 25
Inventive step (IS)	Yes:	Claims	
	No:	Claims	18-23
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.
PCT/EP2005/051464

Re Item II

Priority

The International Searching Authority has not been able to consider the validity of the priority because a copy of the earlier application whose priority has been claimed was not available at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

Should the date of priority claimed not be allowed for the relevant parts of the present application, the documents indicated in the search report as a P-documents would be regarded as state of the art.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Present claims 1-3, 10, 11 and 16 encompass a mutant filamentous fungal cell defined only by a desired characteristic (the mutant filamentous fungal cell has a steering towards homologous recombination, the mutant filamentous fungal cell has a ratio non-homologous recombination/homologous recombination that is decreased or the mutant filamentous fungal cell is deficient in a gene encoding a component involved in non-homologous recombination). Contrary to the requirements of clarity of Article 6 PCT, these claims relate to a result-to-be-achieved type of definition that does not allow the scope of the claim to be ascertained. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claim (PCT Guidelines 9.19 and 9.20). The description provides support and disclosure in the sense of Article 6 and 5 PCT for a mutant in the *hdfA* and/or *hdfB* genes. The search of claims 1-3, 10, 11 and 16 has therefore been restricted to the *hdfA* and/or *hdfB* mutants as disclosed in examples 2, 3 and 4 of the present application. Claims relating to inventions in respect of which no international search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The EPO policy when acting as an International Preliminary Examining

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.
PCT/EP2005/051464

Authority is not to carry out a preliminary examination on matter which has not been searched. Examination will therefore be carried out for the subject-matter of claims 1-3, 10, 11 and 16 when concerning a filamentous fungal cell mutant in the *hdfA* and/or *hdfB* genes.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: TSUKAMOTO Y ET AL: "Hdf1, a yeast Ku-protein homologue, is involved in illegitimate recombination, but not in homologous recombination" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 24, no. 11, 1996, pages 2067-2072.
- D2: BOULTON SIMON J ET AL: "Identification of a *Saccharomyces cerevisiae* Ku80 homologue: Roles in DNA double strand break rejoicing and in telomeric maintenance" NUCLEIC ACIDS RESEARCH, vol. 24, no. 23, 1996, pages 4639-4648.
- D3: BORKOVICH KATHERINE A ET AL: "Lessons from the genome sequence of *Neurospora crassa*: Tracing the path from genomic blueprint to multicellular organism." MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, vol. 68, no. 1, March 2004 (2004-03), pages 1-108.
- D4: DATABASE NCBI NIH; 12 March 2003 (2003-03-12), "*Neurospora crassa* strain OR74A chromosome IV cont3.499" Database accession no. AABX1000206.
- D5: DATABASE NCBI NIH; 12 March 2003 (2003-03-12), "*Neurospora crassa* strain OR74A cont3.5" Database accession no. AABX01000750.
- D6: [Online] 15 March 2005 (2005-03-15), Retrieved from the Internet:

URL:[http://www.fgsc.net/Aspergillus/Asper2 program.pdf](http://www.fgsc.net/Aspergillus/Asper2%20program.pdf) [retrieved on 2005-09-21].

D7: WO 01/68882 A (TRANSKARYOTIC THERAPIES, INC; IVANOV, EVGUENII) 20 September 2001 (2001-09-20).

D8: WO 02/052026 A (UNIVERSITEIT LEIDEN; STICHTING BINAIR VECTOR SYSTEEM; HOOYKAAS, PAUL,) 4 July 2002 (2002-07-04).

Novelty (Art 33(2) PCT

Document D1 discloses the *hdf1* gene that is the *S. cerevisiae* homologue of the DNA sequences 2 and 19 (*hdfA* gene from *A. niger* and *P. chrysogenum* respectively).

Document D2 discloses the *hdf2* gene that is the *S. cerevisiae* homologue of the DNA sequences 5 and 22 (*hdfB* gene from *A. niger* and *P. chrysogenum* respectively).

Document D3 discloses the *N. crassa* homologues of the DNA sequences 2, 5, 19 and 22 (see entries NCU08290.1 and NCU00077.1 in table 24, page 35 and discussion in page 37, right-hand column, third paragraph; see D4 and D5 that correspond to the entries NCU08290.1 and NCU00077.1).

In the light of D1-D5 the subject-matter of claims 24 and 25 is not new.

Document D6 discloses that *N. crassa* disruption mutants *ncku70* and *ncku80*, lacking the *Ku70* and *Ku80* genes, have been obtained and that in these mutants the frequency of homologous integration of ectopic DNA is higher than that of the wild type.

In the light of D6 the subject-matter of claims 1-5, 9-13, 15-17 is not new.

Document D7 discloses a method for improving homologous recombination in an eukaryotic cell, including a filamentous fungal cell, by providing an agent that enhances homologous recombination, for instance the RAD52 protein, or an agent that inhibits non-homologous end joining, for instance an agent that inactivates Ku, such as an anti-Ku antibody or a Ku-binding oligomer or polymer.

In the light of D7 the subject-matter of claims 6-8, 14 is not new.

Inventive step (Art 33(3) PCT)

Document D8 discloses a method for increasing the efficiency of targeted integration of a polynucleotide in an eukaryote by providing an eukaryotic mutant in *ku70* and/or *ku80* genes.

The difference between D8 and the subject-matter of claim 18 is that the method involves, as eukaryotic mutant, a *hdfA* and/or *hdfB* mutant cell from the filamentous fungi *Aspergillus*, *Penicillium* or *Trichoderma*.

In the light of D7 the problem solved by the subject-matter of claim 18 is regarded as the provision of an alternative method for increasing the efficiency of targeted integration of a polynucleotide in an eukaryote by using a *hdfA* and/or *hdfB* mutant eukaryotic cell; the solution being using a *Aspergillus*, *Penicillium* or *Trichoderma* *hdfA* and/or *hdfB* mutant cell.

This solution cannot be regarded as inventive for the following reasons;

D8 discloses that the method can be applied to any eukaryotic mutant in *ku70* and/or *ku80*, including filamentous fungi.

Furthermore D6 discloses that *N. crassa* disruption mutants *ncku70* and *ncku80*, lacking the *Ku70* and *Ku80* genes, have a frequency of homologous integration of ectopic DNA higher than that of the wild type.

It would be obvious for a skilled person in the light of the teaching of D6 and D7 to provide an alternative method for increasing the efficiency of targeted integration that involves a *hdfA* and/or *hdfB* mutant cell from the filamentous fungi *Aspergillus*, *Penicillium* or *Trichoderma* instead of the filamentous fungus *N. crassa*.

In the light of D6 and D8 the subject-matter of claims 18-23 is therefore not inventive.

Re Item VIII

Certain observations on the international application

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/EP2005/051464

Claims 1-3, 9-11 and 16 do not meet the requirements of Art 6 PCT in that the scope of the claim is not clearly defined. The claims are formulated as a so-called result-to-be-achieved. The applicant is requested to introduce the technical features that are responsible for the claimed technical effect to meet the requirements of Art 6 PCT.

PATENT COOPERATION TREATY

REC'D 28 OCT 2005

WIPO PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/EP2005/051464

International filing date (day/month/year)
31.03.2005

Priority date (day/month/year)
02.04.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/90, C12N5/00

Applicant
DSM IP ASSETS B.V.

1. This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the international application
- Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for International preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Guarinos Vifials, E
Telephone No. +49 89 2399-7228



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/051464

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. **type of material:**
 - a sequence listing
 - table(s) related to the sequence listing
 - b. **format of material:**
 - in written format
 - in computer readable form
 - c. **time of filing/furnishing:**
 - contained in the international application as filed.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority for the purposes of search.
3. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. II Priority

1. The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/051464

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

the entire international application,
 claims Nos. 1-3, 10, 11, 16

because:

the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 the claims, or said claims Nos. 1-3, 10, 11, 16 are so inadequately supported by the description that no meaningful opinion could be formed.
 no international search report has been established for the whole application or for said claims Nos.
 the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form has not been furnished

does not comply with the standard

the computer readable form has not been furnished

does not comply with the standard

the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/051464

**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or
industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-17, 24, 25
Inventive step (IS)	Yes:	Claims	
	No:	Claims	18-23
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/EP2005/051464

Re Item II

Priority

The International Searching Authority has not been able to consider the validity of the priority because a copy of the earlier application whose priority has been claimed was not available at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

Should the date of priority claimed not be allowed for the relevant parts of the present application, the documents indicated in the search report as a P-documents would be regarded as state of the art.

Re Item III

Non-establishment of opinion with regard to novelty, Inventive step and industrial applicability

Present claims 1-3, 10, 11 and 16 encompass a mutant filamentous fungal cell defined only by a desired characteristic (the mutant filamentous fungal cell has a steering towards homologous recombination, the mutant filamentous fungal cell has a ratio non-homologous recombination/homologous recombination that is decreased or the mutant filamentous fungal cell is deficient in a gene encoding a component involved in non-homologous recombination). Contrary to the requirements of clarity of Article 6 PCT, these claims relate to a result-to-be-achieved type of definition that does not allow the scope of the claim to be ascertained. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claim (PCT Guidelines 9.19 and 9.20). The description provides support and disclosure in the sense of Article 6 and 5 PCT for a mutant in the *hdfA* and/or *hdfB* genes. The search of claims 1-3, 10, 11 and 16 has therefore been restricted to the *hdfA* and/or *hdfB* mutants as disclosed in examples 2, 3 and 4 of the present application. Claims relating to inventions in respect of which no international search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The EPO policy when acting as an International Preliminary Examining

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.
PCT/EP2005/051464

Authority is not to carry out a preliminary examination on matter which has not been searched. Examination will therefore be carried out for the subject-matter of claims 1-3, 10, 11 and 16 when concerning a filamentous fungal cell mutant in the *hdfA* and/or *hdfB* genes.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: TSUKAMOTO Y ET AL: "Hdf1, a yeast Ku-protein homologue, is involved in illegitimate recombination, but not in homologous recombination" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 24, no. 11, 1996, pages 2067-2072.
- D2: BOULTON SIMON J ET AL: "Identification of a *Saccharomyces cerevisiae* Ku80 homologue: Roles in DNA double strand break rejoining and in telomeric maintenance" NUCLEIC ACIDS RESEARCH, vol. 24, no. 23, 1996, pages 4639-4648.
- D3: BORKOVICH KATHERINE A ET AL: "Lessons from the genome sequence of *Neurospora crassa*: Tracing the path from genomic blueprint to multicellular organism." MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, vol. 68, no. 1, March 2004 (2004-03), pages 1-108.
- D4: DATABASE NCBI NIH; 12 March 2003 (2003-03-12), "*Neurospora crassa* strain OR74A chromosome IV cont3.499" Database accession no. AABX1000206.
- D5: DATABASE NCBI NIH; 12 March 2003 (2003-03-12), "*Neurospora crassa* strain OR74A cont3.5" Database accession no. AABX01000750.
- D6: [Online] 15 March 2005 (2005-03-15), Retrieved from the Internet:

URL:[http://www.fgsc.net/Aspergillus/Asper2 program.pdf](http://www.fgsc.net/Aspergillus/Asper2%20program.pdf) [retrieved on 2005-09-21].

D7: WO 01/68882 A (TRANSKARYOTIC THERAPIES, INC; IVANOV, EVGUENII) 20 September 2001 (2001-09-20).

D8: WO 02/052026 A (UNIVERSITEIT LEIDEN; STICHTING BINAIR VECTOR SYSTEEM; HOOYKAAS, PAUL,) 4 July 2002 (2002-07-04).

Novelty (Art 33(2) PCT

Document D1 discloses the *hdf1* gene that is the *S. cerevisiae* homologue of the DNA sequences 2 and 19 (*hdfA* gene from *A. niger* and *P. chrysogenum* respectively).

Document D2 discloses the *hdf2* gene that is the *S. cerevisiae* homologue of the DNA sequences 5 and 22 (*hdfB* gene from *A. niger* and *P. chrysogenum* respectively).

Document D3 discloses the *N. crassa* homologues of the DNA sequences 2, 5, 19 and 22 (see entries NCU08290.1 and NCU00077.1 in table 24, page 35 and discussion in page 37, right-hand column, third paragraph; see D4 and D5 that correspond to the entries NCU08290.1 and NCU00077.1).

In the light of D1-D5 the subject-matter of claims 24 and 25 is not new.

Document D6 discloses that *N. crassa* disruption mutants *ncku70* and *ncku80*, lacking the *Ku70* and *Ku80* genes, have been obtained and that in these mutants the frequency of homologous integration of ectopic DNA is higher than that of the wild type.

In the light of D6 the subject-matter of claims 1-5, 9-13, 15-17 is not new.

Document D7 discloses a method for improving homologous recombination in an eukaryotic cell, including a filamentous fungal cell, by providing an agent that enhances homologous recombination, for instance the RAD52 protein, or an agent that inhibits non-homologous end joining, for instance an agent that inactivates Ku, such as an anti-Ku antibody or a Ku-binding oligomer or polymer.

In the light of D7 the subject-matter of claims 6-8, 14 is not new.

Inventive step (Art 33(3) PCT)

Document D8 discloses a method for increasing the efficiency of targeted integration of a polynucleotide in an eukaryote by providing an eukaryotic mutant in *ku70* and/or *ku80* genes.

The difference between D8 and the subject-matter of claim 18 is that the method involves, as eukaryotic mutant, a *hdfA* and/or *hdfB* mutant cell from the filamentous fungi *Aspergillus*, *Penicillium* or *Trichoderma*.

In the light of D7 the problem solved by the subject-matter of claim 18 is regarded as the provision of an alternative method for increasing the efficiency of targeted integration of a polynucleotide in an eukaryote by using a *hdfA* and/or *hdfB* mutant eukaryotic cell; the solution being using a *Aspergillus*, *Penicillium* or *Trichoderma* *hdfA* and/or *hdfB* mutant cell.

This solution cannot be regarded as inventive for the following reasons;

D8 discloses that the method can be applied to any eukaryotic mutant in *ku70* and/or *ku80*, including filamentous fungi.

Furthermore D6 discloses that *N. crassa* disruption mutants *ncku70* and *ncku80*, lacking the *Ku70* and *Ku80* genes, have a frequency of homologous integration of ectopic DNA higher than that of the wild type.

It would be obvious for a skilled person in the light of the teaching of D6 and D7 to provide an alternative method for increasing the efficiency of targeted integration that involves a *hdfA* and/or *hdfB* mutant cell from the filamentous fungi *Aspergillus*, *Penicillium* or *Trichoderma* instead of the filamentous fungus *N. crassa*.

In the light of D6 and D8 the subject-matter of claims 18-23 is therefore not inventive.

Re Item VIII

Certain observations on the international application

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/EP2005/051464

Claims 1-3, 9-11 and 16 do not meet the requirements of Art 6 PCT in that the scope of the claim is not clearly defined. The claims are formulated as a so-called result-to-be-achieved. The applicant is requested to introduce the technical features that are responsible for the claimed technical effect to meet the requirements of Art 6 PCT.